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Antimicrobial Resistance of Diarrheagenic *Escherichia coli* Isolated from Children under the Age of 5 Years from Ifakara, Tanzania

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Diarrhea caused by multidrug-resistant bacteria is an important public health problem among children in developing countries. The prevalence and antimicrobial susceptibility of diarrheagenic *Escherichia coli* in 346 children under 5 years of age in Ifakara, Tanzania, were studied. Thirty-eight percent of the cases of diarrhea were due to multiresistant enterotoxigenic *E. coli*, enteroaggregative *E. coli*, or enteropathogenic *E. coli*. Strains of all three *E. coli* categories showed high-level resistance to ampicillin, tetracycline, co-trimoxazole, and chloramphenicol but were highly susceptible to quinolones. Guidelines for appropriate use of antibiotics in developing countries need updating.

Diarrhea caused by multidrug-resistant bacteria has been recognized as an important public health problem among children in developing countries and is a research priority of the diarrheal disease control program of the World Health Organization. Among these bacteria, strains of the different diarrheagenic categories of *Escherichia coli*, such as enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli*, and enteroaggregative *E. coli* (EAggEC), are among the most important causes of acute enteritis and subsequent morbidity and mortality in children in developing countries (4). Knowledge of local antimicrobial therapy patterns is important in selecting the appropriate therapy, since even if microbiology laboratory facilities are available, which is not the case in most of the developing countries, susceptibilities will generally not be known until 72 h after the sample is processed. Acute enteritis is a widespread health problem in these countries and is an important cause of mortality among infants and young children. In Ifakara, Tanzania, children with acute diarrhea are treated by oral rehydration plus administration of an antibiotic (currently co-trimoxazole). Therefore, since many patients with enteritis are treated empirically with antibiotics, it is important to know the antimicrobial resistance patterns of the most prevalent bacteria causing this syndrome.

The main objective of this study was to analyze the prevalence of diarrheagenic *E. coli* as a cause of diarrhea in children under the age of 5 years in Ifakara, Tanzania, as well as to study the susceptibilities of these isolates to six antimicrobial agents.

Stool specimens from 346 children under 5 years of age from Ifakara, Tanzania, who presented with acute diarrhea were cultured for *E. coli* and other enteropathogens by conventional methods in the microbiology laboratory of the Ifakara Center (from April to June 1998). The identified strains were kept on conservation agar. Detection of the virulence factors of *E. coli* strains and susceptibility testing were performed in the clinical microbiology laboratory of the Hospital Clinic, Barcelona, Spain. DNA from each *E. coli* isolate was subjected to PCR under the conditions described in reference 9 to determine the correct diarrheagenic category. The MICs of six antimicrobial agents for the diarrheagenic *E. coli* isolates were determined by the E-test method. Colonies from 24-h McConkey agar cultures were homogenized in 0.85 saline, and the turbidity was adjusted to that of a 0.5 McFarland standard. The inoculum suspension was spread on a Mueller-Hinton agar plate surface with a swab, and after that plates had sat on the bench 15 min, E-test strips were applied. The inoculated medium was incubated for 20 h at 37°C, and the MICs were read. The National Committee for Clinical Laboratory Standards breakpoints were used to differentiate between susceptible and resistant isolates (6). *E. coli* ATCC 25922 was used as a reference strain for quality control purposes. ETEC strains were isolated from 44 children (12.7%). The distribution of these strains according to the type of enterotoxin synthesized was as follows: 33 strains (75%) produced the heat-stable toxin (ST), 6 strains (14%) synthesized the heat-labile toxin (LT), and 5 strains (11%) produced both toxins. EAggEC strains were isolated from 82 (23%) children, and verotoxin-2-producing *E. coli* was detected in one child; no enteroinvasive *E. coli* strains were isolated. Seventeen of these 82 EAggEC strains also produced LT and/or ST. Of these 17 strains, 15 synthesized ST, one strain produced LT, and one strain synthesized both toxins. Finally, EPEC strains were isolated in 21 cases (6%).

In this study, three PCR techniques, amplifying the *bfp* gene, the *eaeA* gene, or the EPEC adherence factor (EAF), were used to detect EPEC strains. Twenty-one strains were found to have at least one of these genes; 13 of the 21 strains had only the *eaeA* gene, 1 strain had only the *bfp* gene, 5 strains were *eae* and *bfp* positive, 1 strain was positive for both *eae* and EAF, and 1 strain was positive for *eae*, *bfp*, and EAF. Therefore, it seems that the presence of the *eae* gene is more highly linked to EPEC than is the presence of either the *bfp* gene or EAF, as has been previously suggested (7).

In this study, ETEC was found to be the category of diarrheagenic *E. coli* which most frequently causes diarrhea in children under 5 years of age, as has been reported for many

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TABLE 1. Antimicrobial susceptibilities of different diarrheagenic *E. coli* isolates from children under 5 years of age in Ifakara, Tanzania

Antimicrobial agent	MICs for and antimicrobial susceptibilities of *E. coli* isolates[a]								
	EAggEC (n = 65)			ETEC (n = 44)			EPEC (n = 21)		
	MIC_{50}	MIC_{90}	No. of resistant strains (%)	MIC_{50}	MIC_{90}	No. of resistant strains (%)	MIC_{50}	MIC_{90}	No. of resistant strains (%)
Ampicillin	>256	>256	54 (83.1%)	>256	>256	37 (84.1%)	>256	>256	19 (90.4%)
Chloramphenicol	>256	>256	37 (57%)	4	>256	11 (25%)	4	>256	7 (33.3%)
Tetracycline	>256	>256	57 (87.7%)	>256	>256	30 (68.2%)	>256	>256	17 (81%)
Co-trimoxazole	>32	>32	59 (90.8%)	>32	>32	35 (79.5%)	>32	>32	19 (90.4%)
Nalidixic acid	6	12	1 (1.5%)	4	12	1 (2.3%)	4	6	0 (0%)
Ciprofloxacin	0.008	0.012	0 (0%)	0.008	0.012	0 (0%)	0.012	0.016	0 (0%)

[a] n, total number of clinical isolates tested.

studies in developing countries (4). Several studies have also shown the importance of EAggEC and EPEC as a cause of diarrhea in children (5). In our study, only one EHEC strain, producing verotoxin-2, was isolated. This result is in agreement with previous studies in which EHEC strains were not found (4). Overall, 38% of diarrhea cases in children under 5 years of age are due to multiresistant diarrheagenic *E. coli*, with ETEC, EAggEC, and EPEC strains being the most prevalent.

Results for the antimicrobial susceptibility testing of the different categories of diarrheagenic *E. coli* strains are shown in Table 1. For all three categories of diarrheagenic *E. coli*, the MICs of ampicillin, tetracycline, and co-trimoxazole at which 50% of the isolates tested were inhibited (MIC_{50}s) were >256 μg/ml. Chloramphenicol showed moderate activity, with resistance ranging from 25 to 57%; the MIC_{50}s of chloramphenicol were 4 μg/ml for ETEC and EPEC and >256 μg/ml for EAggEC. Nalidixic acid and ciprofloxacin had very good activity against these microorganisms; however, one EAggEC strain and one ST-producing ETEC strain showed resistance to nalidixic acid, with MICs of 32 and 256 μg/ml, respectively. Recently, Sang et al. (8) described four cases of diarrhea caused by multidrug-resistant EAggEC in Kenyan children. It was therefore reasonable to predict that this multiresistance showed by the different categories of diarrheagenic *E. coli* might emerge in other developing countries where these classical antibiotics (ampicillin, tetracycline, and trimethoprim-sulfamethoxazole) have been widely used. It has been shown that the treatment of diarrhea caused by ETEC with antibiotics, specifically co-trimoxazole, decreases the duration and intensity of the diarrhea (1). However, in our study, ETEC exhibited high-level resistance to this antimicrobial agent. According to the antibiogram, different resistance patterns were defined (Table 2) in the three categories of diarrheagenic *E. coli*, with Amp^r Cm^s Tc^r Sxt^r Nal^s Cip^s and Amp^r Cm^r Tc^r Sxt^r Nal^s Cip^s being the two most prevalent multiresistance patterns.

TABLE 2. Resistance patterns of the diarrheagenic *E. coli* isolates

Microorganism (n[a])	Pattern	No. of strains with pattern (%)
EPEC (21)	Amp^s Cl^s Tc^s Sxt^s Nal^s Cip^s	1 (4.7)
	Amp^r Cl^s Tc^s Sxt^s Nal^s Cip^s	1 (4.7)
	Amp^r Cl^r Tc^s Sxt^r Nal^s Cip^s	2 (9.5)
	Amp^r Cl^r Tc^r Sxt^r Nal^s Cip^s	5 (23)
	Amp^r Cl^s Tc^r Sxt^r Nal^s Cip^s	12 (57)
EAggEC (65)	Amp^r Cl^r Tc^r Sxt^r Nal^r Cip^s	1 (1.5)
	Amp^s Cl^r Tc^r Sxt^r Nal^s Cip^s	1 (1.5)
	Amp^s Cl^s Tc^s Sxt^s Nal^s Cip^s	2 (3.1)
	Amp^s Cl^s Tc^s Sxt^r Nal^s Cip^s	2 (3.1)
	Amp^s Cl^s Tc^r Sxt^r Nal^s Cip^s	2 (3.1)
	Amp^s Cl^s Tc^r Sxt^s Nal^s Cip^s	4 (6.2)
	Amp^r Cl^s Tc^s Sxt^r Nal^s Cip^s	4 (6.2)
	Amp^r Cl^s Tc^r Sxt^r Nal^s Cip^s	14 (21.5)
	Amp^r Cl^r Tc^r Sxt^r Nal^s Cip^s	35 (53.9)
ETEC (44)	Amp^r Cl^r Tc^r Sxt^r Nal^r Cip^s	1 (2.3)
	Amp^s Cl^s Tc^r Sxt^r Nal^s Cip^s	1 (2.3)
	Amp^r Cl^s Tc^s Sxt^s Nal^s Cip^s	4 (9)
	Amp^s Cl^s Tc^s Sxt^s Nal^s Cip^s	5 (11.3)
	Amp^r Cl^s Tc^s Sxt^r Nal^s Cip^s	5 (11.3)
	Amp^r Cl^r Tc^r Sxt^r Nal^s Cip^s	10 (22.7)
	Amp^r Cl^s Tc^r Sxt^r Nal^s Cip^s	18 (40.9)

[a] n, total number of strains analyzed.

Acute or chronic enteritis due to the different categories of *E. coli*, mainly ETEC and EAggEC, is an emerging problem in many parts of the world (4). It has been estimated that 9.2 million deaths in the developing world have been caused by infectious diseases, and diarrheal diseases are the fourth most prevalent cause (3). Most mild diarrhea cases are successfully managed with oral rehydration therapy. Only for more severe or persistent diarrhea cases should antimicrobial treatment be added. Ampicillin and cotrimoxazole have been recommended by the World Health Organization. Local information about antimicrobial resistance should be used in clinical management, and treatment guidelines should be updated (10). In this sense, ampicillin and co-trimoxazole should be excluded. One alternative for enteritis treatment is the use of quinolones. However, they are not recommended for children. Moreover, it should be taken into consideration that in spite of the minimal use of quinolones in Tanzania, we have detected nalidixic acid-resistant *E. coli* strains. If quinolones are used as a first-line treatment for enteritis in these countries, where the use of antibiotics is not regulated, a rapid emergence of quinolone resistance will likely occur. Rifaximin is a nonabsorbable antimicrobial agent which has been shown to be effective as a treatment for severe episodes of bacterial diarrhea in children in developed countries (2). However, studies to assess the role of this antimicrobial agent in the treatment of ETEC or EAggEC infections in children from developing countries are needed.

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